EEDP-03-2
March 1987

Environmental Effects of Dredging Technical Notes

WETLAND ANIMAL BIOASSAYS/BIOMONITORING

PURPOSE: This note follows Technical Note EEDP-03-1 and adds support to the concept of using wetland animals as indicators of bioavailable contaminants in dredged material used to create intertidal wetlands. The text of this tech note was taken from a paper by Kay, Marquenie, and Simmers (1986).

BACKGROUND: Animal bioassay test procedures are being evaluated (field tested) and verified under the "Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives," called the Field Verification Program (FVP). Bioassays have been shown to be a relatively simple tool for ecological evaluation and environmental assessment of potential contaminant movement within permanent or temporary wetlands, and with field verification, it will be a useful biomonitoring tool as well.

One objective of the FVP was to verify laboratory wetland animal bioassay test results in the field. Lab tests using sediment from Bridgeport Harbor indicated highly toxic effects on the sandworm, *Nereis virens*. Field test results also indicated toxicity. However, the concept of direct verification of laboratory test results in the field proved to be an oversimplification. The goal of confining the sandworm at the FVP wetland site or a reference site and ensuring adequate survival during the field test was not attained. The application of an effective and realistic field test procedure required additional studies to relate the laboratory test species to species actually colonizing the site during the early stages of the wetlands ecological succession.

Current research in the wetland animal bioassay work unit of the FVP suggests that a suite of species occupying different ecological niches in the wetland may be more valuable in bioassay and biomonitoring procedures than a single index species. Different species often bioaccumulate and react to the contaminants in the environment differently; therefore, the usefulness of a bioassay and biomonitoring species may best be evaluated by its capability as an indicator. Some common marine wetland species accumulate metals readily while others tend to accumulate organic contaminants. Literature indicates that a contaminated wetland, such as the FVP wetland site at Bridgeport, Conn., would have a great availability of organic contaminants for the biota and that bioassay/biomonitor species should indicate the potential bioavailability of these contaminants to native species. The mud snail found on the New England intertidal mud flats has been found to be a good indicator of bioavailable polyaromatic hydrocarbons (PAHs), as reported by Kay, Marquenie, and Simmers (1986).

US Army Engineer Waterways Experiment Station, Environmental Laboratory
PO Box 631, Vicksburg, Mississippi 39180-0631

The information herein supports the selection of the mud snail as a complementary bioassay/biomonitor for use in the laboratory and the field. Additional verification of the wetland animal bioassay and biomonitoring procedures will be reported later. The concept presented in this note is the result of ongoing research under the FVP. Draft FVP final guidance on wetland animal bioassay and biomonitoring will be completed in September 1987.

ACKNOWLEDGEMENTS: This report is a product of joint research conducted under the auspices of the United States-The Netherlands Memorandum of Understanding Concerning Dredging and Related Technology. Funding for this study was provided through the Field Verification Program. Laboratory assistance was provided by R. G. Rhett at the Environmental Laboratory, Waterways Experiment Station, and support in analytical chemistry was provided by P. De Jong, H. Gielen, H. Compaan, and J. Spyk, The Netherlands Organization for Applied Research (TNO).

ADDITIONAL INFORMATION OR QUESTIONS: Contact one of the authors, Dr. John W. Simmers (601) 634-2803 (FTS 542-2803), Dr. Stratford H. Kay (601) 634-2387 (FTS 542-2387), Mr. R. Glenn Rhett (601) 634-3717 (FTS 542-3717), or Dr. Charles R. Lee (601) 634-3585 (FTS 542-3585), or the manager of the Environmental Effects of Dredging Programs, Dr. Robert M. Engler (601) 634-3624 (FTS 542-3624).

Introduction

Field verification of the results of laboratory studies is necessary to accurately predict the impact of disposal of dredged material containing complex mixtures of organic and inorganic contaminants (Chapman and Long 1983). Field studies encounter many difficulties that do not exist in the laboratory, including seasonal climatic fluctuations, temporary and extended abnormal weather conditions, predation, and human activities.

The native mud snail, *Ilyanassa obsoleta (Nassarius obsoletus)*, was selected as a potential bioassay animal because of its abundance on the intertidal mud flats of New England. It is one of the dominant invertebrate species found in the Connecticut salt marshes (Brousseau 1981, Fell et al. 1982). It is able to withstand anoxia up to nine days and can partially regulate its oxygen consumption either metabolically or through the formation of oxygen-containing gas bubbles within the mantle cavity (Kushins and Mangum 1971). Mud snails are common on the surface and burrowing within the surficial sediments at low tide in midsummer, thereby demonstrating the ability to withstand the high ambient temperatures of the intertidal mud flat. Therefore, this species is well suited to the prevailing environmental conditions of an intertidal (wetland creation) disposal facility.

Since the mud snail had not been previously used in any bioassay or bioaccumulation studies, it was necessary to determine its suitability as an indicator species by conducting bioaccumulation studies under controlled laboratory conditions. The objective was to assess the potential of the mud snail as an intertidal invertebrate bioassay organism to complement the existing bioassay organism (sandworm) in bioassay and bioaccumulation tests of approximately one month duration.

Materials and Methods

Test sediment was collected from Black Rock Harbor, and mud snails were collected from Tongue Point, both near Bridgeport, Conn. The snails were held in artificial seawater at 20° C and 22 ppt salinity and fed a commercial invertebrate diet.

Laboratory intertidal simulation chambers, 19 × 41 × 12 cm (depth × width × length), were constructed to simulate the effects of exposure to contaminated sediment under conditions of a 12-hr tidal cycle. Each chamber contained 20 ℓ of sediment and was stocked initially with approximately 350 mud snails. Peristaltic pumps gradually inundated the sediment with artificial seawater at 22 ppt salinity over a 6-hr period and drained the sediment in the subsequent 6-hr period. Temperature was maintained constant at 20° ± 1° C throughout the study, and aeration was provided.

In this preliminary study, samples of 50 mud snails each were taken for analysis prior to exposure and at intervals of 4, 8, 16, and 32 days. At the end of each time period, the snails were placed in aerated artificial seawater overnight to purge the gut contents and then were frozen. Sediment and tissue samples were homogenized and extracted, and PAHs were determined by high performance liquid chromatography. Subsamples of about 0.5 g of each tissue homogenate and sediment were used to determine dry (sediment) and ash-free dry (tissue) weights. Due to the preliminary nature of the study, analyses were conducted on only a single sample from each time period.

Results and Discussion

As indicated, the bioaccumulation of specific PAHs either appeared to plateau during the first 8 to 16 days or continued to increase throughout the

study (Figure 1). After 32 days, benzo(a)pyrene and benzo(k)fluoranthene continued to increase. Chrysene uptake had plateaued by day 16. Pyrene, fluoranthene, and benzo(a)anthracene declined after an initial period of accumulation. The uptake of pyrene, fluoranthene, chrysene, and benzo(a)anthracene was rapid during the first 8 days; whereas uptake of benzo(a)pyrene and benzo(k)fluoranthene appeared to be slow and continuous throughout the study. This preliminary time-series experiment with the mud snails suggested that a 1-month exposure would be adequate to attain the steady state for some lower and intermediate molecular weight PAHs desired in the bioassay. The initial rapid increase of several PAHs followed by a sharp decline after 16 days was similar to that demonstrated previously for phenanthrene in a detritus-feeding clam, *Macoma balthica*, and in a burrowing polychaete, *Abarenicola pacifica* (Augenfeld et al. 1982). The relatively high concentrations of some PAHs in the snails may be the result of a relatively poorly developed mixed-function oxidase (MFO) enzyme system in the snails, as only recently have MFO enzyme systems been detected in gastropods (Payne and May 1979).

Those PAHs that accumulated to the highest concentrations in the snails were the lower molecular weight compounds, both more water soluble and more easily degraded metabolically than the higher molecular weight compounds. This agrees with one report (Mix and Schaffer 1983) that the low molecular weight unsubstituted PAHs bioaccumulate to higher levels than the higher molecular weight compounds. The sampling technique used in the study disturbed the sediment each time snails were collected, thus mixing the deeper strata with the top 1 to 2 cm of sediment. Each disturbance possibly released a pulse of contaminants, causing a shift toward a new steady state. The concentrations of the more soluble PAHs increased rapidly following each disturbance of the sediment. When the time between sampling periods was constant, uptake of the more soluble PAHs was rapid and essentially linear. As the time between sampling periods lengthened, the more labile PAHs apparently were lost rapidly through metabolic activity and passive elimination. These results agree with the statement of Kveseth, Sortland, and Bokin (1982) that the lower molecular weight compounds may depurate more readily than the higher molecular weight compounds.

The more persistent PAHs such as benzo(a)pyrene continued to bioaccumulate for a much longer time, but more slowly than compounds such as pyrene and

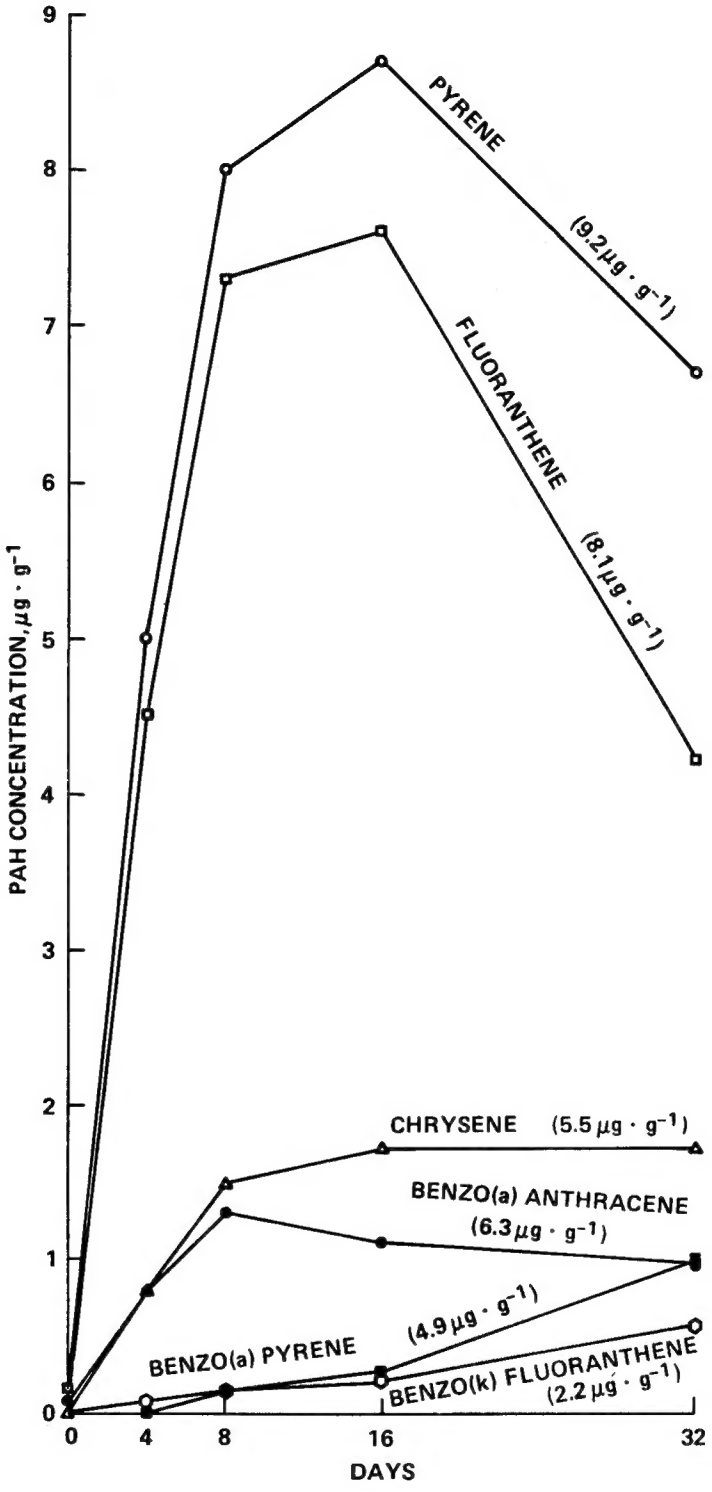

Figure 1. Time-dependent uptake of PAH by mud snails. Values in parentheses are PAH concentrations in the sediment

fluoranthene. The net result of the competing processes of bioaccumulation and elimination ultimately would be greater accumulation of the less soluble, higher molecular weight PAHs over a longer period of time. Consequently, under field conditions, the higher molecular weight PAHs could bioaccumulate to higher concentrations than the lower molecular weight PAHs. This factor must also be considered in the interpretation of bioassay test results.

This preliminary study suggests that the mud snail may be a good indicator of long-term bioaccumulation of many PAHs, especially those that are more persistent. The use of this species to assess bioaccumulation of the more water-soluble PAHs may be limited due to the possible rapid elimination of these compounds. These results also demonstrate the necessity for extreme care in the design of time-course studies involving bioaccumulation of contaminants from sediment. The mud snail is well suited as a biomonitor/bioassay animal, although further research is needed before the mud snail bioassay can be applied beyond the New England area to predict accurately the potential steady-state conditions in an intertidal disposal site.

References

Augenfeld, J. M., Anderson, J. W., Riley, R. G., and Thomas, B. L. 1982. "The Fate of Polyaromatic Hydrocarbons in an Intertidal Sediment Exposure System: Bioavailability to *Macoma inquinata* (Mollusca:Pelecypoda) and *Abarenicola pacifica* (Annelida:Polychaeta)," Marine Environmental Research, 7:31-50.

Brousseau, D. J. 1981. "Gametogenesis and Spawning in a Population of *Geukensia demissa* (Pelecypoda:Mytilidae) from Westport, Connecticut," Veliger, 24:247-251.

Chapman, P. M., and Long, E. R. 1983. "The Use of Bioassays as a Part of a Comprehensive Approach to Marine Pollution Assessment," Marine Pollution Bulletins, 14:81-84.

Fell, P. E., Olmstead, N. C., Carlson, E., Jacob, W., Hitchcock, D., and Silber, G. 1982. "Distribution and Abundance of Macroinvertebrates on Certain Connecticut Tidal Marshes, with Emphasis on Dominant Molluscs," Estuaries, 5:234-239.

Kay, S. H., Marquenie, J. M., and Simmers, J. W. 1986. "Time Dependent Uptake of PAHs by Mud Snails, *Ilyanassa obsoleta*," p. 70-72, In Proceedings of 2nd International Conference on Environmental Contamination, CEP Consultants Ltd., Edinburgh, UK.

Kushins, L. J., and Mangum, C. P. 1971. "Responses to Low Oxygen Conditions in Two Species of the Mud Snail *Nassarius*," Comparative Biochemistry and Physiology, 39A:421-435.

Kveseth, K., Sortland, B., and Bokin, T. 1982. "Polycyclic Aromatic Hydrocarbons in Sewage, Mussels and Tap Water," Chemosphere, 11:623-639.

Mix, M. C., and Schaffer, R. L. 1983. "Concentrations of Unsubstituted Polynuclear Aromatic Hydrocarbons in Bay Mussels (*Mytilus edulis*) from Oregon, USA," Marine Environmental Research, 9:193-209.

Payne, J. F., and May, N. 1979. "Further Studies on the Effect of Petroleum Hydrocarbons on Mixed-Function Oxidases in Marine Organisms," In Khan, M.A.Q., Lech, J. J., and Menn, J. J., eds., Pesticide and Xenobiotic Metabolism in Aquatic Organisms, American Chemical Society, Washington, DC, pp. 339-347.